

## CLAIMS

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A13
1. A method of selection of a nucleic acid encoding an enzyme that is capable of converting a prodrug to its active drug form comprising the steps of:
- a) contacting a population of bacteria transformed with a bacteriophage library with a prodrug in a medium, wherein;
    - i) the transformed bacteria are in the lysogenic state, and
    - ii) when converted to its active drug form, the prodrug causes activation of the proteolytic activity of bacterial RecA and lysis of the bacteria;
  - b) separating bacteriophage particles released by lysis of the bacteria from said medium, and
  - c) analysing the genotype of said separated bacteriophage for a nucleic acid encoding the enzyme.
2. A method for selection of a nucleic acid encoding an enzyme capable of converting a prodrug to its active drug form comprising the steps of:
- a) introducing a library of genes into bacteriophage to form a bacteriophage library;
  - b) infecting a population of bacteria with said bacteriophage library;
  - c) selecting said infected bacteria for bacteria in which the lysogenic state has been established;
  - d) contacting said bacteria with said prodrug in a medium;
  - e) separating from said medium bacteriophage particles released by lysis of host bacteria; and
  - f) analysing the genotype of said selected bacteriophage for the nucleic acid encoding the enzyme;
- wherein said prodrug causes activation of the proteolytic activity of bacterial recA when converted to its active drug form.
3. A method according to claim 1 or claim 2 wherein said steps are repeated in at least one cycle.
4. A method according to any one of the preceding claims wherein the DNA sequence of said released bacteriophage particles is analysed.

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5. A method according to any one of the preceding claims wherein said bacteriophage carry a gene encoding antibiotic resistance or other selectable marker.
6. A method according to any one of the preceding claims wherein said enzyme is nitroreductase, flavin reductase, DT-diaphorase, thymidine kinase, cytosine deaminase or a purine nucleoside phosphorylase.
7. A method according to any one of the preceding claims wherein said prodrug is CB1954, SN 23862, 2-[N,N-bis(2-iodoethyl)amino]-3,5-dinitrobenzamide 5-fluorocytosine, acyclovir, ganciclovir, or 6-methyl-9-(2-deoxy- $\beta$ -D-erythro-pentofuranosyl) purine.
8. A method according to any one of the preceding claims wherein said temperate bacteriophage is the bacteriophage lambda or a lambda derivative.
9. A method according to any one of claims 2-8 wherein said gene library comprises genes encoding variants of a single enzyme.
10. A method according to claim 9 wherein said variants comprise amino acid deletions, insertions and substitutions from the wild type enzyme sequence.
11. A method according to claim 9 or 10 wherein said variants are generated by DNA shuffling, random mutagenesis, or PCR shuffling.
12. A method according to any one of the preceding claims wherein said activity of said bacterial RecA protein is caused by the generation of single-stranded DNA in the bacterium.
13. A method according to claim 12 wherein said single-stranded DNA is generated as a consequence of the enzymatic conversion of prodrug to its active drug form.
14. A method according to claim 13 wherein said single-stranded DNA arises as a result of a break in one or both strands of the DNA, a cytotoxic lesion, a DNA crosslink or a monovalent DNA adduct, or by inhibition of the progress of DNA replication by any other means.
15. A method according to any one of the preceding claims wherein said enzyme comprises nitroreductase and said prodrug comprises CB1954.

16. A method according to any one of the preceding claims wherein said bacteriophage vector is  $\lambda$ JG3J1.
17. A method according to any one of the preceding claims wherein said bacterium is of the *E. coli* strain C600Hfl.
18. A catalytic enzyme or enzyme fragment isolated according to the method of any one of the preceding claims.
19. A nucleic acid molecule encoding a catalytic enzyme or enzyme fragment according to claim 18.

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